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THE NEGLECTED FIELD OF HEAT LOSS

When the student of nutrition is taught the oxidation of a foodstuff such as carbohydrate the reaction is usually written

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O.$$

This neglects the chief product of the reaction, heat or energy. When he is taught excretion there is a long discussion of the nitrogenous substances and little or no attention is given to the elimination of heat.

The question arises as to whether the study of elimination of heat belongs in the field of nutrition. As a rule it is scarcely mentioned in modern textbooks or journals devoted to this subject. Long ago it received full attention from Lavoisier, Rubner, Atwater, Benedict, and Lusk, but it has been crowded out by the growing interest in vitamins and minerals. Heat production still holds a fairly respectable place in the literature. Does heat elimination belong to another discipline? Should it be relegated to other journals and textbooks?

Heat elimination must in the long run balance heat production. Under certain conditions such as cold weather or sleep or disease it may actually determine the level of heat production. With animals the environmental temperature has a profound effect on the resting metabolism. Men have a fairly wide temperature range in which the metabolism is basal. Women have a narrower zone. Variations in clothing may change materially the level of heat production and yet relatively little is known about the scientific basis for the insulating effects of clothing. How does a suit of clothes affect the relative proportions of heat lost by radiation and convection? How can we provide troops in the Arctic and aviators with materials that will keep them from shivering? There have been no significant discoveries in the field of clothing since the dawn of history. Probably the best and most scientific cold weather clothing is that worn by the Eskimos who still have the culture of the late stone age.

The literature on nutrition pays relatively little attention to the question of internal temperature. Many students of the subject still hold to the idea that the temperature of the human body is accurately represented by the little arrow on the clinical thermometer. They recognize the effects of temperature changes on the rate of chemical reactions *in vitro;* they neglect the fact that the different parts of the human body have many different temperatures and that the temperature of any one part varies with the time of day and with the degree of activity. How many of our students realize that most of our athletic records are made by men with fever temperatures caused by the discrepancy between heat production and heat loss?

It may be asked if nutrition as a discipline should try to cover a wide field with outlying areas that may belong in part to physiology or biophysics. Nutrition cannot afford to abandon territory that belonged to it in the great period of its development. It must include not only the study of heat production and heat elimination but also intermediary metabolism, tissue metabolism, and a great deal of biochemistry as well as food economics. If it neglects these border zones it may become so narrow that it will have to change its name to dietetics.

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MATERNAL NUTRITION AS RELATED TO PREGNANCY AND FETAL DEVELOPMENT

The effect of maternal nutrition on the course of pregnancy and labor has received considerable emphasis during recent months (*Nutrition Reviews* **1,** *276 (1943)*). Attention has been given to the relationship of diet to the occurrence of polyneuritis, the frequency of anemia during pregnancy, and the character of labor. There has been less adequate analysis of the effects of maternal nutrition on the health of the fetus even though Hart, McCollum, Steenbock, and Humphrey (*Res. Bull. no. 17, Wis. Agr. Exp. Sta. (1911)*) many years ago pointed out that the diet of heifers seriously affected the viability and vigor of calves, and Warkany (*Nutrition Reviews* **1,** *207 (1943)*) more recently has demonstrated that the diet fed to breeding female rats influenced the incidence of congenital abnormalities in their offspring. Of great interest, therefore, is the report by Burke, Beal, Kirkwood, and Stuart (*Am. J. Obst. Gyn.* **46,** *38 (1943)*) of a long study of the effect of maternal nutrition on fetal development and infant health as well as on the complications of pregnancy.

The women selected for the investigation were patients in the prenatal clinics of the Boston Lying-in Hospital. They were examined at frequent intervals throughout pregnancy and careful dietary analyses were made. Diets were called optimal if they provided daily 2600 to 2800 calories, 85 to 100 g. of protein, 1.5 g. calcium, 2.0 g. phosphorus, 20 mg. iron, 8000 I.U. vitamin A, 2.0 mg. thiamine, 2.5 mg. riboflavin, 18 mg. niacin, 100 mg. ascorbic acid, and 400 to 800 I.U. vitamin D. A rating of "excellent" was assigned to those diets which contained these "optimal" amounts of foodstuff, "good" if they had 80 per cent or more, "fair" if they contained 60 per cent to less than 80 per cent, "poor" if they provided less than 60 per cent, and "very poor" if they contained less than 50 per cent. The observations reported were obtained on 216 women and their infants. The majority of the families represented had weekly incomes of $25 to $40. About 70 per cent of the women were under the age of 30 years.

A statistically significant relationship was found to exist between the adequacy of the maternal diet and the course of pregnancy. Of the women with "good" or "excellent" diets, 68 per cent had a normal prenatal course, while of the women with "poor" to "very poor" diets, only 42 per cent had a normal prenatal course. Furthermore, no instance of preeclampsia occurred in those women whose diets were "good" or "excellent," but almost 50 per cent of the mothers whose diets were "poor" to "very poor" had toxemia. No statistically valid correlation could be made between the nutritional adequacy of diets consumed and length and character of labor or the over-all complications of labor and delivery. Mothers whose diets were "poor" to "very poor" tended to have more difficult types of labor and more major complications at delivery despite the fact that they had, on the average, smaller infants than were born to the women in the "good" or "excellent" diet group. There was a tendency for a relationship to exist between prenatal nutrition and major complications of the postpartum period, but the relationship was not definite or distinct.

The influence of maternal diet on the condition of the infants was striking. Mothers whose nutrition was "good" or "excellent" gave birth to children with pediatric ratings as follows: 42 per cent "superior," 55 per cent with one or two minor physical defects, and 3 per cent with congenital defects. On the other hand, infants born of mothers on "poor" to "very poor" diets were rated: 2.5 per cent "superior," 2.5 per cent with one minor physical defect, 28 per cent in fair or poor general condition, and 67 per cent in "poorest" condition. This latter group consisted of

infants who were stillborn, who died within a few hours or days, had marked congenital malformations at birth, were premature (under 5 pounds at birth), or were "functionally immature." The size of the infants was equally striking. Those born of mothers with the best diets had an average weight of 8 pounds 8 ounces and an average length of 20.4 inches; those born of mothers with inadequate diets averaged only 5 pounds 13 ounces in weight and 18.6 inches in length. In only one instance did a mother whose nutrition was good give birth to an infant in poor physical condition.

This correlation was made on the basis of maternal nutrition as related to the condition of the infant. If the process is reversed and the condition of the infant at birth is correlated with the adequacy of the maternal diet, the results are equally convincing. The "superior" infants were produced by mothers whose diets had the following percentage distribution: "good" or "excellent" 56, "fair" 35, and "poor" to "very poor" 9. In contrast, when the condition of the "poorest" infants was studied in relation to the prenatal nutrition, it was found that 79 per cent of the mothers had diets which were rated as "poor" to "very poor," 18 per cent had "fair, "and only 3 per cent had "good" or "excellent" diets.

From these results it appears that poor nutrition during pregnancy affects the fetus more profoundly than it does the maternal organism. This is contrary to the usual obstetric teaching, and should certainly give both direction and impetus to the prenatal health program now spreading throughout the nation. The authors state that the physical status of the women during pregnancy was evaluated and, since the study was painstakingly done, it is probably safe to assume that women selected for the investigation suffered no evident impairment of health other than the conditions listed. The critical reader, however, would like to have had additional data in regard to the nutritional status of the women during pregnancy, namely hemoglobin and red blood cell counts, serum proteins, and plasma ascorbic acid, which might have added to the validity of the dietary histories.

HISTOPATHOLOGY OF SKIN IN AVITAMINOSIS A

Frazier and Hu (*Arch. Int. Med.* **48,** *507 (1931)*) described cutaneous lesions in patients they observed in China which they attributed to vitamin A deficiency. Later Rao in India (*Indian J. Med. Res.* **25,** *39 (1937)*) recorded similar observations and conclusions. The most characteristic feature of the lesions was the formation of keratotic plugs in the hair follicles, with a rough dry skin resulting; hence such titles as phrynoderma, "toad skin" and "nutmeg grater skin." However, it has been difficult to accept the conclusions which the authors drew because the diets of the subjects of these reports were deficient in more than one respect. Furthermore, little had been reported experimentally which could support these observations in man. Wolbach and Howe (*J. Exp. Med.* **42,** *753 (1925)*) reported slight, although demonstrable atrophy of hair follicles and sebaceous glands in association with vitamin A deficiency, but they did not observe distended keratinized follicles. Portman (*Acta path. et microbiol. Scandinav.* **4,** *341 (1927)*) described the loss of subcutaneous fatty tissue and a reduction in the total number of hair follicles present.

In what appears to be a careful systematic study in rats, Moult (*Arch. Dermat. Syph.* **47,** *768 (1943)*) has presented experimental evidence which gives support to the observations made in man. The rats, obtained at weaning, were fed a diet consisting of coconut cake meal, 30 per cent; flaked rice, 38 per cent; casein (ashless, extracted),

10 per cent; dried yeast, 8 per cent; wheat germ, 5 per cent; peanut oil (refined), 5 per cent; and salt mixture, 4 per cent. Vitamins A and D were given separately, the latter in doses of 1 I.U. per day for all rats. The dosage of vitamin A ranged in different animals from 50 to 0 I.U. per day. The animals were killed at different ages. Small areas of skin were excised from the regions of the abdomen, thorax, shoulder, and foot-pad of each animal.

The illustrations which Moult publishes give evidence of striking differences in the appearance of sections of skin from rats fed the various quantities of vitamin A. In animals given 50 I.U. per day, the cells of the epidermis showed a well graded progression from the cells of the malpighian layer to the thin layer of the stratum corneum, and the elements of the dermis were well formed. There were abundant stores of both interfollicular and subdermal fat, and the hair follicles were numerous and in close juxtaposition. The cells at the root of each follicle appeared in a state of active proliferation, and the sebaceous glands were normal.

In rats fed only 20 I.U. of vitamin A per day, the hair follicles were less numerous and not so well developed as those described above. In rats fed only 10 I.U., the fat stores were not so abundant as in the previous groups, and hyperkeratosis was found to occur more frequently.

In rats fed 5 I.U. per day, the hair follicles were moderately numerous one month after weaning, but there was a high proportion of distended follicles and some decided hyperkeratosis. In animals of the same series allowed to live for three months after weaning, an excessive degree of hyperkeratosis was found at the orifices of some hair follicles, and there was a reduction in the amount of interfollicular fat.

In rats fed only 2 I.U., the whole skin was thinner, and the abnormalities described above were more marked and were visible at an earlier age. In some of these animals the mouth of the hair follicle was considerably distended and the keratin material protruded beyond the surface of the skin, closely investing the issuing hair.

In rats given only 1 I.U. of vitamin A and in other animals given none whatever, changes were still more pronounced. In the last group, even one week after weaning, the degree of follicular hyperkeratosis was advanced to such an extent that the upper thirds of some follicles were already distended by an excessive keratinization. In older animals, keratotic plugs were found.

These studies indicate that in vitamin A deficiency there is first a slight general or diffuse hyperkeratosis, which is followed by an increased keratinization of the epithelium forming the upper third of the hair follicle. Subsequently, this follicular hyperkeratosis becomes so intense that the orifices of the follicle may become partly distended with a bulbous mass of keratin, although the hair itself continues to grow and the sebaceous gland may be healthy. Further increase in the degree of keratinization consists of a decided distention of the follicular orifices and impedes both the growth of the hair and the outflow of sebum. Eventually a distinct plug, consisting of a stratified mass of keratin, is formed. There is a progressive diminution in the thickness of the skin and in the size and number of hair follicles in animals fed progressively smaller quantities of vitamin A. The fat stores are completely lost and the sebaceous glands become atrophic. These changes are observed at an earlier age in animals fed the lowest quantities of vitamin A.

Moult distinguishes between the primary lesions for which the absence of vitamin A is specifically responsible, and the secondary sequelae. He believes that the excessive keratinization of epithelial tissues is a direct result of vitamin A deficiency. In a state of partial deficiency, he noted a considerable reduction in the total number of

hair follicles. The actual cessation of growth of hair, however, he believes is due to purely mechanical obstruction caused by the incipient plugging at the mouth of each individual hair follicle. Similarly, interference with the outflow of sebum probably induces atrophy of the sebaceous glands. The data which Moult presents give convincing evidence of the validity of his conclusions.

PECULIARITIES OF RUMINANT NUTRITION

From the standpoint of digestion, ruminating animals have presented intriguing and baffling problems to the bacteriologist, physiologist, and nutrition worker. Furthermore the adult ruminant presents problems entirely different from those which are characteristic of young ruminants. An excellent review on the nutrition of calves has been presented by Savage and McCay (*J. Dairy Sci.* **25,** *595 (1942)*). Under the title "Some Peculiarities of Ruminant Nutrition," Goss of the University of California (*Nutrition Abst. and Rev.* **12,** *531 (1943)*) has brought together most of the important literature dealing with the nutrition of the mature ruminant.

In his introductory remarks Goss distinguishes between the four compartment stomach of the young calf and that of the adult ruminant by pointing out that in the calf the first three compartments are relatively small whereas the abomasum or true stomach occupies the greater part of the abdominal cavity. As the animal matures the rumen increases in size while the true stomach remains comparatively small. During this transformation and the consequent adjustment to bulky forage crops, many types of microorganisms become established in the rumen or paunch. It must be borne in mind that the first three compartments, rumen (or paunch), reticulum (or honeycomb), and omasum (or manyplies) possess no secretory function and that only the abomasum contains glands capable of producing gastric juice. Consequently any chemical changes which occur in the first three compartments of the ruminant stomach must, of necessity, be brought about chiefly by microorganisms and their accompanying enzyme systems. While the ruminant secretes large amounts of saliva, much of which passes to the rumen, there is little evidence that it exerts any enzymatic effect on feedstuffs. Some writers believe that bovine saliva possesses mild lipolytic properties.

The chemical changes brought about during rumen fermentation are by no means inconsiderable. Such changes seem to be largely fermentative in character. Goss dercsibes the various technics used in studying chemical changes in the rumen and points out that much progress has been made in obtaining qualitative data. The lack of quantitative information is largely due to the fact that liquid materials are leaving the rumen continuously. Not until some method is found by which the rumen can be closed, preventing the passage of food particles to the true stomach, will it be possible to obtain quantitative data of real value. If this could be done, it would then be possible to use a permanent rumen fistula through which samples could be removed for chemical examination. Goss explodes the idea, held for many years by livestock men and early research workers, that ruminating animals cannot live on cereal concentrates alone and that roughage is required to keep such animals alive. In support of this statement he cites his own experimental work in which calves were reared to 19 months of age without roughage, and heifers were kept for 6 years on a ration consisting of concentrates supplemented with cod liver oil and calcium carbonate. The addition of paper pulp, as an artificial

roughage, had no effect on rumination or digestion. However, concentrated rations had to be fed often and in small amounts to prevent bloating. He states that gases formed during bloating consisted of carbon dioxide and air with no detectable methane.

Goss presents an interesting discussion of the researches leading to the discovery that ruminants possess the ability to synthesize many of the essential vitamins in the rumen through the agency of microorganisms. All available evidence points to the fact that most of the members of the vitamin B-complex are synthesized in the rumen. The vitamins which increase during rumen fermentation are thiamine, riboflavin, pyridoxine, biotin, and pantothenic acid. Recent evidence indicates that nicotinic acid is also synthesized to some extent. Little is known regarding the effect of one vitamin on the microbial synthesis of other vitamins but there is reason to believe that this is a factor that must be taken into account in future studies on vitamin synthesis in the digestive tract.

No evidence exists to indicate the synthesis of ascorbic acid in the rumen. In fact work at California and Pennsylvania shows that ascorbic acid is destroyed in the rumen although it would appear that ascorbic acid is synthesized by the dairy cow, probably in the body tissues. The mechanism of this synthesis is unknown. Vitamins A, D, and E are not synthesized by ruminants and must be furnished in the feed. Vitamin K, however, seems to be produced liberally as a result of rumen fermentation.

Goss gives an excellent résumé of recent work on conversion of nonprotein nitrogen to protein in the ruminant. Particular emphasis is placed on work at Wisconsin and Illinois showing that urea can be utilized by microorganisms to synthesize protein (protoplasm) which can be utilized eventually by the ruminant. Johnson, Hamilton, Mitchell, and Robinson (*J. Animal Sci.* **1,** *236 (1942)*) have advanced the interesting theory that when the protein level in the ration is approximately 12 per cent, much of the protein utilized by the ruminant comes from protoplasmic material obtained from microorganisms growing profusely in the rumen. Experiments are now in progress to test this hypothesis. It is interesting that many rations, including the ration supplemented with urea, yielded biologic values close to 60. It is only when urea and ammonium salts replace a part of the protein in rations incapable of maintaining nitrogen equilibrium, that positive results are obtained. When protein is high and adequate, it is impossible to show that urea is utilized.

The ruminant is unique in that the four compartment stomach makes it possible to ingest amounts of roughages which are too bulky for other types of livestock. From these roughages the ruminant is able to extract about 30 per cent of the available energy. This is accomplished by digestion of fibrous materials through the agency of microorganisms and these organisms in turn are able to synthesize many essential vitamins as well as proteins.

The ruminating animal offers a challenge to the physiologist, the biochemist, the bacteriologist, and the nutrition worker. Before much more progress can be made the veterinary surgeon should pave the way for future research by finding operative procedures which will make possible the study of rumen chemistry and biology uncomplicated by the passage of rumen contents to the true stomach during normal rumen fermentation.

CORRELATION OF DIETARY FINDINGS WITH NUTRITIONAL STATUS

Dietary surveys do not, by themselves, provide a direct and accurate measure of the prevalence of malnutrition (*Nutrition Reviews* **1**, *72 (1942)*). Detailed examination of individuals for functional and anatomic changes resulting from inadequate consumption or utilization of essential elements of the diet should constitute an important part of such surveys. In most instances, it is necessary to confirm the clinical observations by therapeutic trials with adequate amounts of the food factors considered to be lacking, and to provide placebo controls as a check for therapeutic treatments. However, due to the vagueness and inadequacy of many of the signs and symptoms ascribed to mild deficiencies, the nonspecificity of others, and the shortage of clinical examiners trained and experienced in modern technics of studying nutritional deficiency diseases of the milder sort, public health workers in the field of nutrition can be guided by the results obtained from carefully conducted dietary surveys.

The lack of correlation which may be found to exist between dietary findings and simultaneous observations of nutritional status is well illustrated in the report by Riggs and co-workers (*Canad. J. Pub. Health* **34**, *193 (1943)*). These workers procured dietary records, physical examinations, medical histories, dental examinations, and certain laboratory tests on 260 boys and 286 girls between the ages of 11 and 19 years, in the Collegiate Institute, East York Township, Canada. For the diet studies, the students recorded in small booklets their total daily food consumption during one week, with a description of the sizes of servings. The physical examination included all the points recommended by the National Research Council (*Pub. Health Reports* **57**, *189 (1942)*) for studies of nutritional status. Laboratory and other tests included determinations of blood hemoglobin, routine urinalysis, slit lamp examination of the eye for conjunctival lesions and corneal vascularization, measurements of capillary fragility by the suction-cup procedure, and dark adaptation tests with the Wald-Stevens portable adaptometer.

The adequacy of the diet was determined by comparison with the recommended daily allowances of the Food and Nutrition Board of the National Research Council. Values for individual factors were expressed as percentages of the recommended daily allowances and classified as follows: "Excellent, 90 per cent or more of the standard; good, 80–90 per cent; fair, 70–80 per cent; poor, below 70 per cent." Substantial proportions of the boys' diets were rated poor in calories (12.3 per cent), calcium (22.7 per cent), thiamine (70.4 per cent), riboflavin (23.8 per cent) and ascorbic acid (80.4 per cent); among the girls' diets, 24.9 per cent were poor in calcium content, 11.5 per cent in iron, 56.0 per cent in thiamine, 16.8 per cent in riboflavin, and 74.8 per cent in ascorbic acid. The diets were also rated for protein and vitamin A contents, which were found to be poor in only a small proportion, for both boys and girls.

In contrast to these dietary findings, little evidence of malnutrition and none of the recognized signs of specific vitamin deficiencies were found. Where it was possible to compare variations from the normal, detected on physical examination, with the intake of dietary factors known to be among the causes of such variations, little correlation was found. For example, "thirty per cent of the girls had weights below normal and three-fifths of this 'underweight' group had calorie intakes which were above 90 per cent of the standard. In the case of the boys, 22 per cent were judged as underweight and one-half of these had excellent supplies of calories." Of those with the smallest supplies of thiamine, 40 per cent of

both the girls and boys had a food intake which rated excellent in calories. More than half the students who had weights above normal had low intakes of this vitamin. Part of the explanation of the failure to find evidences of thiamine deficiency may lie in the high fat content of the diets. On the average, these students secured about 45 per cent of their total calories from fat. For the years 1935 to 1939, fat provided 36 per cent of the total calories in the United States food supply. It has been repeatedly demonstrated that the thiamine requirement varies directly with the number of calories obtained from carbohydrate sources. Estimations of the thiamine requirements of individuals should therefore be based upon their consumption of carbohydrate, if accuracy is desired. However, recommendations based on total caloric intakes are more practical when dealing with large population groups and permit a more satisfactory margin of safety.

High blood hemoglobin values were recorded for all the girls whose diets had been graded poor in iron content. Some 44 per cent of the girls and 59 per cent of the boys showed conjunctival changes, described as indicative of vitamin A deficiency, in spite of the finding that 86.8 per cent of the girls' and 84.7 of the boys' diets were graded excellent in vitamin A content. No impairment of dark adaptation was found. No relation was found between the recorded dietary content of vitamin A and the incidence of colds reported by the students.

Comparisons were made between calcium intake and the number of active dental caries, calcium intake and the number of dental fillings, calcium intake and height. No correlation was found in any of these comparisons. A comparison was also made of the dental conditions in those students who were taking an extra source of vitamin D with those without such a supply. No protection against caries was evident. Fifty per cent of all subjects examined had more than four active caries. These observations merely tend to confirm those of numerous other investigators on the multiplicity of responsible factors in the production of dental caries.

Although fully cognizant of the weaknesses inherent in "diet scores," Riggs *et al.* used this device "to evaluate the composite value of the food consumption." Their justification for this appears to be that such a composite rating is desirable and the method used by them is as fair as any other. Actually the pedagogic trick of assigning so many credits to each component and thereby arriving at a grade for the whole has no applicability in situations where each and every part of the whole is essential; a chain is as strong as its weakest link. The fallacy of "diet scores" is obvious when one compares the record that only 6.6 per cent of the girls and 3.1 per cent of the boys had poor "diet scores" with the incidence of specific dietary deficiencies given above.

Each examining physician was requested to grade each student as excellent, good, fair, or poor with regard to nutritional status. Fifty-two per cent of the girls and 65 per cent of the boys were graded excellent, 26 and 12 per cent good, 20 and 22 per cent fair, and 2 and 1 per cent respectively, poor. It would appear from this, that in spite of the absence of clearcut signs of specific vitamin and mineral deficiencies and in spite of the authors' failure to demonstrate correlations between nutritional status and the dietary intake of specific food factors, a large proportion of the students were not enjoying optimum nutrition in the opinion of the examining physician. Our standard for normalcy should be nothing less than "excellent."

THE NUTRITIVE VALUE OF DEHYDRATED VEGETABLES

The subject of dehydration of foods is under intensive investigation. The rewards, particularly in time of war, are apparent: many foods may be reduced to a fifth or tenth of their fresh weight and volume and hence save greatly in shipping and storage space, and in container materials. It is not a simple problem, however, to provide a dehydrated vegetable which upon rehydration will possess a reasonable degree of original appearance, taste, and nutritive quality. Some of the dehydrated soups offered to the public are palatable, but here the use of flavors and condiments may offset other possible flavor changes occurring during dehydration. Many of the dehydrated vegetables are certainly less desirable from the esthetic standpoint. It is these considerations which are most likely to determine whether dehydrated vegetables will or will not assume a general public use after the war. At the present time it seems unlikely that they will find any great role in home consumption, but they are of immediate importance in the war program and merit intensive study.

Fundamental in the objects of the dehydration program is the question of the preservation of the vitamins which are present in the fresh product. Such factors as the type and extent of blanching before dehydration, the temperature, time and degree of drying, and storage conditions, are prime considerations in attaining maximum stability of the vitamins. A preview of the work in this field reveals that there are few general principles to follow since each vitamin and even each vegetable presents a different problem. The data with respect to vitamins A and C are commonly in disagreement, but many apparent differences must depend on the different processing conditions employed by various investigators.

Work by Beckley and Notley (*Biochem. J.* **35,** *1396 (1941)*) on the preservation of ascorbic acid in vegetables indicated that a lower dehydration temperature, even though requiring a longer time, was more favorable. Bruising of the vegetables prior to dehydration was found to yield a product inferior in vitamin C content. It was reported preferable to blanch potatoes and cauliflower before dehydration, while carrots, green beans, and cabbage should be dried without previous blanching. It was also noted that vegetables which were not blanched before dehydration might lose even more vitamin C upon soaking due to reactivity of oxidative enzymes. The vitamin C loss on dehydration, however, was great with all the vegetables, amounting to 70 to 90 per cent, and no principle was evolved as to how to preserve this unstable vitamin.

Moyer (*J. Am. Dietet. Assn.* **19,** *13 (1943)*) has reported results from the New York State Agricultural Experiment Station on the nutritive value of vegetables at different stages of the dehydration process. In the case of vitamin C it was a general finding that 20 to 30 per cent may be lost during blanching and that a further great loss occurred during the actual drying. Other reports indicate a fair percentage of retention as the result of a lower temperature of dehydration (less than 70°C.); nevertheless nearly complete losses occurred during storage. The most recent results confirm earlier conclusions that dried fruits and vegetables are nearly devoid of vitamin C. Even fresh vegetables as commonly prepared, with the exception of tomatoes, cannot be relied upon to furnish any large proportion of the daily vitamin C requirement.

Of all the vitamins, we rely most heavily on vegetables for our source of vitamin A, mostly in the form of carotene. Here, as in the case of vitamin C, there is present in certain vegetables an enzyme which may destroy the vitamin. The data concerning

vitamin A losses during drying are neither complete nor in general agreement. Moyer reached the conclusion that little carotene is lost during the processing of rutabagas prior to dehydration or in the actual drying process. It was not completely stable during storage but the losses could be minimized by storage in an atmosphere of carbon dioxide.

The vitamin A values of dehydrated carrots and sweet potatoes are of particular interest since they are rich sources of carotene. A Puerto Rican variety of sweet potato grown in Texas is now being commercially dehydrated and marketed as "Vita Yam." Earlier results of Fraps and Treichler (*J. Agr. Research* **47,** *539* (*1933*)) indicated considerable loss of vitamin A due to oxidation during the drying process. Scoular and associates (*J. Am. Dietet. Assn.* **19,** *428* (*1943*)) noted very little loss of carotene in the sweet potato during drying and only a 17 per cent loss during storage for one year in a metal container at room temperature. Lease and Mitchell (*S. Carolina Agr. Exp. Sta. Bull. no. 329* (*1940*)) report that the carotene content of raw Puerto Rican sweet potato averages 40 micrograms per gram and the flour 130, which with a water content of the fresh product of 68 per cent, indicates no loss of the provitamin during the dehydration process.

The situation appears to be different in the case of carrots since Scoular and associates confirmed earlier work in finding an 80 per cent loss of carotene during drying. Likewise in the case of spinach, DeFelice and Fellers (*Proc. Am. Soc. Hort. Sci.* **35,** *728* (*1937*)) noted at least a 90 per cent loss of the original vitamin A activity as a result of dehydration.

Few values are to be found in the literature for the B-vitamin contents of dehydrated vegetables. Moyer's study indicates an average loss of thiamine of 20 to 25 per cent during dehydration processing of cabbage, rutabagas, beets, and potatoes. This is due largely to the leaching action during blanching. Thiamine was stable during storage for a six month period whether the vegetable was packed in tightly closed glass containers, under carbon dioxide in closed glass containers, or in cellophane or pliofilm bags. Other B-vitamins, with the possible exception of riboflavin, would be expected to withstand the drying process and be stable in a variety of storage conditions.

The preservation of vitamins during dehydration and storage of vegetables is obviously a complex problem. From the strictly nutritional standpoint we rely on the vegetable group as a source of carbohydrate, minerals, vitamin A, and in some cases, protein. Carbohydrate, minerals, and proteins are largely retained during dehydration, but in the case of vitamin A it would appear that only certain vegetables retain a high proportion during dehydration and storage, and others do not. Much more work is therefore warranted on this specific subject. From a more general standpoint, a liberal quantity and variety of vegetables constitutes a more acceptable dietary for the average American, and hence contributes to the morale of the soldier. At least for the duration of the war, therefore, great emphasis will necessarily be placed on the development of dehydrated vegetables, irrespective of their vitamin contents. It is nevertheless the nutritionist's and food technologist's wartime duty to find the conditions for the maximum preservation of nutrients in these foods.

EXPERIMENTAL MANGANESE DEFICIENCY

Manganese is one of the trace elements essential to the maintenance of normal nutrition in animals. It is needed for normal reproduction, lactation, bone formation, and

growth (Shils and McCollum, *J. Am. Med. Assn.* **120,** *609* (*1942*)). There has, however, been considerable disagreement among various workers as to the exact manifestations of manganese deficiency in rats and mice. For instance, workers at the University of Wisconsin have reported decreased growth and disturbances of the estrus cycle resulting in sterility (Boyer, Shaw, and Phillips, *J. Biol. Chem.* **143,** *417* (*1942*)), while Orent and McCollum (*J. Biol. Chem.* **92,** *651* (*1931*)) found no differences in estrus or growth but noted poor reproduction with high mortality of the young and poor lactation performance. Daniels and Everson (*J. Nutrition* **9,** *191* (*1935*)) obtained still different results; in their experiments there was a high mortality of the young animals as a result of congenital debility, but no disturbances of lactation or estrus. Shils and McCollum (*J. Nutrition* **26,** *1* (*1943*)) have recently reported an extensive study of manganese deficiency in rats and mice which was undertaken in an attempt to explain some of the contradictory results of earlier work.

Shils and McCollum fed their animals a solid basal diet low in manganese (0.2 to 0.3 microgram per gram) which had the following percentage composition: whole milk powder, 20; cottonseed oil U.S.P., 11.68; casein and dried yeast extract, 16; sucrose, 45; and a salt mixture, 2.82. Supplements were given to provide, per kilogram, 20 drops of percomorph oil, 200 mg. of choline, and 1 mg. of thiamine chloride. The drinking water was distilled and contained 1 ml. of 8 per cent potassium iodide solution per liter. The average manganese intake of an adult rat on this diet was less than 3 micrograms per day. When the ration was supplemented with manganous chloride at a level of 0.18 per cent, it supported excellent growth.

Young female rats fed the deficient diet for 2 months and then mated produced roughly the same number of litters as did a control group given the same diet with added manganese. However, only 1.5 per cent of the young born of deficient females were weaned. The behavior of the deficient mothers was normal in that they attempted to care for their young and were able to raise young rats transferred to them from normal females. When manganese was given to the deficient mothers and new litters produced, half of the young were weaned. It is evident, therefore, that manganese is necessary for the production of viable young and that this aspect of manganese deficiency is reversible. There was no significant difference between the deficient and the control groups with respect to fertility or the estrus cycle. This result is different from that obtained by Boyer, Shaw, and Phillips. The authors point out that Boyer *et al.* used a milk diet lower in manganese, and suggest that the consequent more acute manganese deficiency may have caused the disturbance in estrus cycle noted in those studies.

Males born of mothers given the deficient diet during pregnancy were sterile and showed testicular atrophy. But young male rats born of normal mothers and fed the deficient diet from the time of weaning showed no differences from controls in growth, sperm motility, or testicular weight over a period of fourteen months.

That manganese deficiency can be produced in adult females was shown by placing 11 stock females, 5 to 6 months of age, on a deficient diet near the end of a pregnancy. All the young appeared normal and were weaned. Continued mating of these adult females on the deficient diet resulted in increasingly poor viability of their offspring.

The development of these young rats, and of those in the 1.5 per cent group which were weaned from the first experiment described above, was abnormal. Growth rate was poor, particularly among the males. Shortening and bowing of the forelegs were noted as gross skeletal abnormalities. The animals also frequently showed an ataxia, incoordination, and loss of equilibrium. Occasionally the affected animal walked in an

essentially normal manner but soon fell over on its side or back and righted itself with difficulty. These symptoms increased in severity to about the eighteenth day and then the animals either died or recovered to a great extent. Since no definite pathologic changes were observed, the authors assumed that an acute "biochemical lesion" of some sort had been produced by the deficiency. A similar ataxia in the offspring of manganese deficient hens has been observed by Caskey and Norris (*Proc. Soc. Exp. Biol. Med.* **44**, *332* (*1940*)).

The poor growth rate referred to in the preceding paragraph had, as is stated, specific reference to animals born of manganese deficient mothers. No decrease in growth was noted in the deficient rats of the first generation on the basal diet. However, when the calcium and phosphorus contents of the basal diet were increased, a marked decrease in growth rate occurred even among first generation males. Calcium and phosphorus behave similarly in intensifying the symptoms of manganese deficiency in chicks (Wilgus and Patton, *J. Nutrition* **18**, *35* (*1939*)). It is suggested that these two elements make the manganese of the ration less available.

Arginase activity determinations made on 14 deficient and 13 control rats showed: (1) a decrease of approximately 50 per cent in the arginase activity in the livers of deficient rats, (2) that addition of manganese raises the activity in the deficient group to that of the controls, and (3) that arginase activity in the females is lower than that in the males of both groups. Although arginase is important in the formation of urea, Shils and McCollum, in confirmation of earlier work, could find no change of the nitrogen excretion in manganese deficiency.

Similar experiments with white mice were reported to give results entirely comparable to those of rats.

This study demonstrates how complex the manifestations of manganese deficiency in rats are, and emphasizes how little is known about the fundamental biochemical abnormalities which are responsible for the changes. Shils and McCollum could not confirm the observations of other workers that deficient mothers behave abnormally toward their young or do not suckle normal young rats. There are no other essential points of difference which cannot logically be fitted into their conclusion that three distinct stages of manganese deficiency may be developed in the female. In the least severe stage, the deficient female gives birth to viable young which develop symptoms of ataxia and poor equilibrium. In the second stage, nonviable young are born. In the most severe depletion, the estrus cycle is disturbed and sterility results.

DIET AND STOMACH LESIONS

There is considerable evidence that feeding nutritionally incomplete diets may result in gastric abnormalities, although the mechanism of the reaction involved has not been fully elucidated, and there is still question whether factors other than diet might also be operative in the formation of the lesions. In 1913 Singer observed ulceration of the epithelium of the forestomach of rats fed bread and wood shavings. Pappenheimer and Larimore (1924) observed stomach lesions on diets composed mainly of white flour, and while the main cause of the lesions was said to be dietary, part of the effect was also attributed to irritation from ingested hair. Fibiger (1919) fed rats and mice a suboptimal diet plus cockroaches infested with Spiroptera, and observed lesions which he attributed to the irritating action of these worms. However, recent investigators regard the deficient diet as a major contributing factor in his experiments.

Other workers have employed more drastic means for producing irritation in the stomach. A celluloid ball covered with pin points or pieces of cork with protruding pig bristles was introduced. Chemical irritation has been produced by a piece of rubber sponge impregnated with Scharlach R or with pine tar oil. Recently Sugiura (*Cancer Research* **2**, *770* (*1942*)) produced gastric ulceropapillomas in rats fed a diet of polished rice, carrots, and kieselguhr.

On the other hand much evidence suggests that ulceration of the forestomach may result from the feeding of a deficient diet alone, without any additional irritating factor. The diets employed usually consist of fortified white flour which is still incomplete from a nutritional point of view. Lesions have been reported in rats deficient in any one of the following factors or combinations: protein, vitamin A, the vitamin B-complex, cystine, choline, riboflavin, pyridoxine, and pantothenic acid. Nevertheless the suggestion has been advanced that an additional irritating factor is also operative in these pure deficiencies. Presumably both the pepsin and the hydrochloric acid in the empty stomach tend to act corrosively. Also, the incidence of gastric lesions is decidedly enhanced as the result of periodic starvation. Another physiologic factor may be regurgitated bile. Sharpless (*Cancer Research* **3**, *108* (*1943*)) fed a borderline diet with and without the addition of sodium taurocholate, and observed that this salt increased the incidence of lesions on the poor diets although it caused no macroscopic changes in rats fed a stock ration. A mixture of pepsin and hydrochloric acid acted like the sodium taurocholate.

In other experiments Sharpless observed that on diets deficient in choline and other factors, regurgitated intestinal contents were in the stomachs of a large percentage of the animals. There was also invariably a high percentage of gastric lesions. In the presence of choline, on the other hand, no intestinal bile was observed in the stomach, and gastric lesions were almost entirely absent. As a matter of fact the animals often exhibited a slight diarrhea. Hence the suggestion was made that the choline had functioned by stimulating the movement of materials through the gastrointestinal tract, and thus minimized the chances of regurgitation and exposure of the gastric mucosa to bile. On the other hand, the well known action of choline as a dietary essential and donator of methyl groups suggested that choline might have some intrinsic merit in maintaining the integrity of the stomach lining.

Recently Sharpless and Sabol (*J. Nutrition* **25**, *113* (*1943*)) studied the effects of specific vitamins when added to a basal mixture of white flour, salts, butterfat, riboflavin, thiamine, and viosterol. The incidence of gastric lesions was 63 per cent on this mixture, and pyridoxine failed to reduce the number of lesions. However, the addition of choline lowered the incidence of lesions to 31 per cent and the mixture of choline plus pyridoxine lowered it to 14 per cent. Additional calcium pantothenate failed to alter this latter result. Thus in these experiments also, choline appeared to be of great importance in the prevention of ulcerous lesions of the forestomach in rats.

PROTEIN METABOLISM AND THE VITAMIN B-COMPLEX

There is convincing evidence to indicate that a relationship between protein metabolism and the vitamin B-complex exists. As long ago as 1925 it was observed that rats grow poorly when fed an increased amount of protein without being provided with a greater supplement of the B-complex (Hartwell, *Biochem. J.* **19**, *1075* (*1925*); Reader

and Drummond, *Biochem. J.* **20,** *1256 (1926)*). A short time later Tscherkes (*Biochem. Z.* **167,** *203 (1926)*) demonstrated that the development of B-avitaminosis in rats is accelerated by diets high in protein. More recently Conger and Elvehjem (*J. Biol. Chem.* **138,** *555 (1941)*) found that the dermatitis of pyridoxine deficiency could be produced more readily with diets that contained 30 per cent casein than when lower amounts of protein were fed. The nature of the relationship between protein metabolism and the B-vitamins, however, is not well understood.

Kleiber and Jukes (*Proc. Soc. Exp. Biol. Med.* **49,** *34 (1942)*) provided the first definite evidence that components of the B-complex favorably influence protein anabolism. They found the protein content of chicks deficient in riboflavin to be less than that of pair-fed controls.

Using an entirely different method of study, Sarett, Klein, and Perlzweig (*J. Nutrition* **24,** *295 (1942)*) also concluded that riboflavin, niacin, and pyridoxine may stimulate the anabolism of protein. These workers studied the urinary excretion of the above three vitamins in dogs and rats maintained on diets which provided a constant amount of the B-complex but a varied amount of protein. Their experimental ration contained mixtures of purified casein, beef suet, corn starch, sucrose, salt mixture, cod liver oil, and dried brewers' yeast. The protein was varied so as to provide, in three different diets, 2.6 per cent, 17.6 per cent, and 41 per cent of the total calories taken. Compensating adjustments were made in the fat and carbohydrate. Feeding was controlled so that the caloric and vitamin intake per unit of weight were equalized.

The authors state that reciprocal changes in the fat and carbohydrate of the diets did not affect urinary excretion of the vitamins; data supporting this statement were not given. The excretion of riboflavin and total niacin by dogs was highest during periods of low protein intake. Change to the high protein diet was associated with a change in nitrogen balance from negative to positive and a decrease in excretion of both vitamins. The changes in riboflavin excretion occurred promptly while those of niacin were more gradual. Similar results were obtained with riboflavin excretion in the experiments on rats; the variations in urinary niacin output, however, were irregular, presumably because rats synthesize niacin. On the last day of each experimental period, dogs were given subcutaneously a test dose consisting of 0.22 mg. of riboflavin, 2.2 mg. of niacinamide, 0.11 mg. of thiamine, and 0.22 mg. of pyridoxine. The per cent of the test dose of riboflavin excreted by all dogs was highest on the low protein diet and lowest on the high protein diet. Where a difference in response to the test dose of niacin (4 out of 5 dogs) or pyridoxine (3 out of 5 dogs) was obtained, the per cent of the test dose excreted was also highest on the low protein diet. The authors believe that the increased retention of riboflavin, niacin, and pyridoxine on the high protein diets is an accompaniment of protein anabolism since there was positive nitrogen balance and often a gain in weight. To show that this increased retention was not caused by protein catabolism, they studied the excretion during periods of starvation and periods during which glycine was substituted for casein in the ration. Under both sets of circumstances urinary output of niacin and riboflavin was increased.

It is interesting that Sarett and associates found that variations of protein intake effected no change in the urinary excretion of thiamine. This result is consistent with the observations of Banerji that protein has a sparing action on thiamine (*Biochem. J.* **35,** *1354 (1941); Nutrition Reviews* **1,** *172 (1943)*).

In a continuation of this work, Sarett and Perlzweig (*J. Nutrition* **25,** *173 (1943)*) studied the concentration of riboflavin, niacin, and protein in the carcasses of rats fed the following four types of diets: high

protein-high vitamin, high protein-low vitamin, low protein-high vitamin, and low protein-low vitamin. Diets were prepared as in the previous experiment. The two levels of protein intake were 40 per cent and 8 per cent, respectively. The high vitamin ration provided daily the extract from 5 g. of fresh liver plus 0.5 mg. of thiamine, 0.5 mg. of riboflavin, 1.65 mg. of niacin, 0.05 mg. of pyridoxine, and 1 mg. of calcium pantothenate. The low vitamin ration contained 40 per cent of these amounts. Six rats were included in each group. Half were sacrificed at twelve days and half at twenty-six days. The concentrations of riboflavin and niacin found in the carcasses (intestinal tract and liver removed) were approximately the same in all four groups of animals. However, in the liver the concentrations of riboflavin and niacin were high with the high protein diets, low with the low protein diets. These changes were independent of the vitamin intake. The thiamine content of the tissues did not vary with any of the diets but the nitrogen content of both liver and carcass was higher with the high protein ration than with the low and was independent of the vitamin intake. These experiments strongly suggest that protein deposition is necessary for the retention of riboflavin and niacin by the liver.

Other investigators have studied the relationship of protein and the vitamin B-complex in an entirely different way: by measuring the effect of specific deficiencies on nitrogen metabolism. Griffith and Mulford (*J. Nutrition* **21,** *633* (*1941*)), for instance, found that a deficiency of dietary choline and of the labile methyl supply in young rats produced a marked elevation of the nonprotein nitrogen of the blood. This effect, however, may have been secondary to renal hemorrhagic degeneration. Schaefer, McKibbin, and Elvehjem (*J. Biol. Chem.* **143,** *321* (*1942*)) reported that, in dogs with a severe state of pantothenic acid deficiency, there is a rise in the blood nonprotein nitrogen.

Sure and Ford (*J. Nutrition* **24,** *405* (*1942*)) more recently studied the nitrogen partition of the blood and urine of albino rats made deficient in thiamine, riboflavin, pyridoxine, and pantothenic acid. Animals were pair-fed to equalize the plane of nutrition. The experimental diets were made from mixtures of purified casein, agar-agar, butterfat, hydrogenated cottonseed oil, salts, sucrose or dextrin or cerelose, and cod liver oil. Supplements of the crystalline vitamins of the B-complex, or of autoclaved yeast or rice polish factor II were made, subject to the restrictions imposed by the particular deficiency state being produced. Blood samples from the thiamine deficient rats and from their controls were collected at weekly intervals for seventy-seven days. In the deficient animals, there were significant increases in blood nonprotein nitrogen (40 per cent or more) and in blood urea nitrogen (about 24 per cent). However, since no microscopic renal lesions were demonstrated at necropsy, it is possible that a part or all of these changes may have been caused by anhydremia. There were also increases in the preformed creatinine, but no significant changes in uric acid and creatine. Urine analysis showed that large amounts of ammoniacal nitrogen were excreted and a marked creatinuria developed. The excretion of creatinine plus its retention in the blood may be correlated with muscular atrophy during thiamine deficiency while the increased output of ammoniacal nitrogen may be explained by the attempt to neutralize acids developed in the acidosis associated with avitaminosis B_1. The findings in riboflavin deficiency were less remarkable. There were no noteworthy variations in nonprotein nitrogen of the blood. Urine analysis showed a moderate creatinuria and marked excretions of ammonia. The explanations offered for these changes are similar to those for the thiamine

depleted animals. Nine pairs of rats were made deficient in pyridoxine and were followed for seventy-six days. They all showed a marked retention of blood creatine and uric acid, a reduction in the excretion of uric acid, and a mild creatinuria. Sure concludes that these results suggest both a disturbance of renal function and an abnormality of muscle metabolism in pyridoxine deficiency. In the rats with pantothenic acid deficiency, the only significant rise in blood nonprotein nitrogen and urea occurred terminally. A moderate creatinuria developed. The one change, therefore, which was constant for all four types of deficiency was a creatinuria.

From these accumulated data it would appear that: (1) some of the components of the B-complex have a favorable influence on protein anabolism, (2) protein seems to spare thiamine, (3) protein deposition may be necessary for the retention of riboflavin and niacin by the liver, and (4) a creatinuria develops during deficiencies of thiamine, riboflavin, pyridoxine, and pantothenic acid, presumably because of the attendant protein catabolism. The biochemical explanations for these relationships have not been furnished.

TYROSINE POISONING IN RATS

Even though it does not appear to be of immediate importance from a nutritional standpoint, it is interesting to note that ill effects have been reported to follow the administration of large amounts of the amino acid, tyrosine. It has long been recognized that urinary excretion of tyrosine occurs in association with severe liver disease, but this has been regarded as a result of marked impairment of liver function. Baer and Blum (*Arch. f. exper. Path. u. Pharmakol.* **56,** *92* (*1907*)) regarded tyrosine as one of the four amino acids (leucine, isoleucine, tyrosine, and phenylalanine) responsible for the formation of ketone bodies. Bickel (*Klin. Wchnschr.* **18,** *178* (*1939*)) reported that tyrosine prevents the accumulation of glycogen in the liver.

Abderhalden (*Ztschr. f. physiol. Chem.* **77,** *454* (*1912*)) reported that 50 to 150 g. of tyrosine could be taken within one day without any apparent ill effects. Later Medes (*Biochem. J.* **26,** *917* (*1932*)) administered 5 to 10 g. and occasionally as much as 50 g. to normal persons without their experiencing untoward reactions. Newburgh and Marsh, however, (*Arch. Int. Med.* **36,** *682* (*1925*)) reported renal injury in dogs and rabbits when they were given tyrosine in doses of 2 g. per kilogram of body weight. Lillie (*Pub. Health Reports* **47,** *83* (*1932*)) also reported minor tubular degenerations of the kidney, exudative blepharitis, and red edematous swelling of the extremities in rats kept for as long as twenty days on a basal diet containing 20 per cent of tyrosine. Recently Hueper and Martin published an extensive report on "Tyrosine Poisoning in Rats" (*Arch. Path.* **35,** *685* (*1943*)).

Rats receiving an adequate diet were given 10 parts of l-tyrosine in place of an equivalent amount of sucrose, making a total of 11.17 parts of tyrosine in the diet. Within one week, the animals had crusted eyelids, opacities and ulcers of the cornea, and swollen red legs and feet. The majority of the rats died within three weeks, while a few survived almost five weeks.

Two main types of pathologic changes were observed, namely, (1) those of a degenerative and necrotizing nature, affecting the parenchyma of the pancreas, eye, kidney, brain, liver, spleen, testes, and marrow; and (2) those of the circulatory-vascular type, as represented by congestion, hemorrhages, edema, perivascular fibrosis, and arteriolar medial degeneration affecting the brain, lungs, heart, adrenal glands, kidneys, and

marrow. The most striking changes were found in the pancreas.

These changes are much more extensive than have hitherto been described and require further study as to their relationship to the administration of tyrosine. If the changes described were actually due to the action of tyrosine, it is of great interest to note that a normal constituent of protein, for which there is a definite nutritional and metabolic need, can produce ill effects when it is given in great excess of the physiologic need. The observations are also of interest because the administration of tyrosine has been recommended (Abelin, *Klin. Wchnschr.* **14,** *1777 (1935)*) in the treatment of hyperthyroidism as a detoxicant of the thyroid hormone.

THE MICROBIOLOGIC DETERMINATION OF RIBOFLAVIN

The Snell and Strong microbiologic assay for riboflavin (*Ind. Eng. Chem. (Anal. Ed.)* **11,** *346 (1939)*) has been employed successfully by many laboratories on a large variety of biologic materials. However, it has been recognized that the microbiologic method gives higher results than other methods on certain types of material, notably cereals and cereal products. A further report by Strong and Carpenter (*Ind. Eng. Chem. (Anal. Ed.)* **14,** *909 (1942)*) describes a comparative study of modified procedures proposed for the riboflavin assay, a study of the effect of pure fatty acids, glycerides, and other organic materials on the test organism, and a simple and effective procedure which has been developed for determining riboflavin in cereal products and other materials. It is concluded that the substances present in cereals and certain other biologic materials which interfere with the determination of riboflavin by the microbiologic method are probably small amounts of free fatty acids. Methods for avoiding their effect have been presented.

According to the authors, it would seem that the preparation of cereals and certain other samples for microbiologic riboflavin assay should consist of two main steps: (1) an hydrolysis, and (2) a procedure designed to remove fat soluble interfering substances. The hydrolysis should be sufficiently drastic to break up various combined forms of riboflavin and thus reduce the problem to the determination of a single compound. The removal of interfering substances from the hydrolysate can be accomplished successfully in most cases by a careful filtration. Ether extraction has been found equally satisfactory, but is usually complicated by emulsion formation if much solid matter is present. In any event, it is time-consuming in routine work, and has been an adjunct in only a few cases where the filtration alone proved inadequate. The validity of these procedures is supported by (1) lower and more reasonable results on mixed feeds and composited diet samples, which by the direct procedure have often appeared to contain twice as much riboflavin, or more, than the sum of the constituents; (2) absence of drift at different dosage levels; (3) satisfactory recovery of added riboflavin; and (4) good agreement with the results of independent assays by other methods on the same samples.

This paper raises a question concerning the accuracy of riboflavin values previously obtained by the original microbiologic method. It appears probable that on samples of relatively high potency, the older values are substantially correct. However, results obtained by the direct method on cereals, mixed feeds, composited diets, fish meals, milk, meat, and certain other materials are probably too high.

A direct comparison has been made of the riboflavin content of various materials determined by the original procedure and by the modified procedure. Whole wheat flour

yielded 1.3 micrograms per gram when assayed by the modified procedure and 2.5 micrograms per gram when determined by the original method, while whole wheat bread gave 1.8 micrograms per gram by the modified method as compared to 2.7 micrograms per gram by the original method. Whole milk assayed by the modified procedure contained 1.7 micrograms per gram and by the original method 2.1 micrograms per gram. The riboflavin content of ground beef determined by the original method was 3.9 micrograms per gram versus 1.3 micrograms per gram when determined by the modified procedure. Ice cream yielded 2.0 micrograms per gram with the modified procedure and 7.7 micrograms per gram using the original method. Whole oats gave 1.7 micrograms per gram by the modified procedure and 3.7 micrograms per gram by the original method. Other materials showing marked differences in riboflavin content when the modified procedures are employed include yellow corn, whole barley, wheat gray shorts, wheat bran, meat scraps, fish meal, soybean meal, and corn gluten meal.

Wegner, Kemmerer, and Fraps (*J. Biol. Chem.* **146,** *547 (1942)*) have reported a similar study, using the same modifications in technic. Again, a direct comparison of the riboflavin content of various materials determined by the original procedure and by the modified procedure was made, and results comparable to those of Strong and Carpenter were obtained. Food materials in which there were notable differences in the results obtained by using the modified procedures were meat and bone scraps, cottonseed meal, whole wheat flour, wheat gray shorts, rice bran, pinto beans, whole barley, whole oats, peanut meal, fish meal, soybean oil meal, corn gluten feed, and tankage.

A more recent study in which further modifications have been employed in the extraction procedure is that of McIntire, Schweigert, Henderson, and Elvehjem (*J. Nutrition* **25,** *143 (1943)*). This paper presents a study of the effects of cooking on the retention of vitamins in pork and has been reviewed previously in this journal (*Nutrition Reviews* **1,** *281 (1943)*).

As cereal products, meat, and milk are among the major sources of riboflavin in the American diet, the new data on riboflavin assays are of especial interest to those concerned with the appraisal of the riboflavin content of the diet and its correlation with laboratory and clinical findings.

GEOGRAPHIC FACTORS AND VEGETABLE NUTRIENTS

Attempts to study the various factors affecting the synthesis of specific chemical compounds in plants are fraught with almost insurmountable difficulties, since it is physically impossible to control but a few of the many factors affecting the formation and deposition of these compounds in plants. Nevertheless, agricultural scientists are continuously attempting to find answers to questions of this type because of the practical uses to which such knowledge can be put in our national nutrition and food production program.

It is common knowledge that the vitamin content of certain crop plants varies and that these variations may be caused by innumerable factors such as soil, climate, fertilizer treatment, time and amount of rainfall, and many others, including varietal and genetic factors.

A recent attempt to contribute further data toward the solution of this problem has been made by Reder of Oklahoma, Ascham of Georgia, and Eheart of Virginia who have attacked the fertilizer problem from a regional or geographic standpoint (Reder, Ascham, and Eheart, *J. Agr. Research* **66,** *375 (1943)*). Using a single variety of

turnip greens as the experimental food plant, these workers have standardized methods of planting and fertilizer applications in such widely separated agricultural areas as Virginia, Oklahoma, and Georgia. The object of the study was to determine, if possible, the factors influencing the synthesis of ascorbic acid in turnip leaves. Soils were analyzed, meteorologic data were recorded, and these data were treated statistically. Nitrogen fertilization results were not clear-cut, the effect of place being more important, apparently, than nitrogen treatment. Potassium fertilization tended to decrease the ascorbic acid content of turnip greens regardless of the district in which they were grown. The greatest decrease of ascorbic acid, as a result of potash treatment, occurred in Georgia. The following averages of large numbers of samples, expressed as mg. per g. of ascorbic acid, give some idea regarding the effect of "place" on the vitamin C content of turnip greens: Norfolk, Va. 2.41; Blacksburg, Va. 1.28; Stillwater, Okla. 1.59; and Experiment, Ga. 1.91. Potash fertilization reduced the amount of ascorbic acid at all of the aforementioned places. These reductions were also variable and ranged from .0102 to .1927 mg. per g. of fresh material. It should be pointed out that workers in this field are not in complete agreement regarding the influence of potassium salts on vitamin synthesis in plants. No significant data were obtained so far as phosphorus fertilization was concerned.

Comparisons of data on soil composition, temperature, and ascorbic acid content of leaves yielded no significant results. Some evidence was obtained to indicate that fall greens were richer in ascorbic acid than greens grown in the spring. No conclusions were drawn regarding the effect of rainfall, although places having the lowest rainfall produced spring greens containing the highest amount of ascorbic acid. Where rainfall and sunshine were at a maximum, ascorbic acid synthesis was found to occur at a relatively low rate as measured by the ascorbic acid content of the harvested plants.

In many, if not most, experiments of this type the effect of "place" seems to be more important than many so-called controlled factors. No one, as yet, has been able to evaluate the intangible variables that contribute to the unquestioned influence of "place" on plant growth and metabolism.

THIAMINE INACTIVATION BY RAW FISH

It is now well established that certain uncooked fish contain a factor or factors which possess the unique property of inactivating thiamine. Foxes which received diets containing 10 per cent or more of fresh fish became afflicted with a characteristic paralysis which became known as Chastek's disease, a paralysis which bears a resemblance to Wernicke's disease in man (*Nutrition Reviews* **1**, *61* (*1942*)).

This subject may not appear to have a direct bearing on human nutrition since it is unlikely that many people are fond of eating uncooked fish. Nevertheless the subject has found wide interest, and it has already been suggested that the thiamine-destroying factor may find extensive laboratory use in the preparation of thiamine free diets as a substitute for prolonged autoclaving. The function of this factor in the physiology of the fish is also unknown, but it is not impossible that the factor, if an enzyme, may be utilized for the synthesis of thiamine in this species.

Recent papers have revealed several facts as to the nature and distribution of the thiamine-inactivating factor. First of all, it seems certain that the paralysis syndrome is due to a lack of thiamine. Green, Carlson, and Evans (*J. Nutrition* **21**, *243* (*1941*)) demonstrated great differences in the thiamine requirement of foxes depending on

whether raw fish was included in the diet. They found that diets containing 20 per cent of raw carp had no ill effects if the diet contained 10 mg. or more of thiamine. The onset of paralysis was delayed at least a month if as little as 2 mg. of thiamine were added to the diet. In analytical experiments, using either the thiochrome or the yeast fermentation method for thiamine, it has also been shown that fresh fish destroys thiamine. In feeding practice it appears that the destruction of thiamine takes place in the feed mixture, prior to ingestion.

It has been a general finding that cooked fish is innocuous in the production of the paralysis syndrome. Woolley (*J. Biol. Chem.* **141,** *997 (1941)*), in studying the heat stability of the inactivating factor, noted that when carp suspensions were heated to boiling and cooled, about one-half the activity of the factor remained. Autoclaving at 15 pounds pressure destroyed all the antithiamine factor. Woolley further noted that the factor was (1) not extracted with ethanol, (2) that suspensions of the fresh carp tissue showed greater activity than water extracts, and (3) that the factor could be extracted with 10 per cent sodium chloride but the active material was not dialyzable.

Sealock, Livermore, and Evans (*J. Am. Chem. Soc.* **65,** *935 (1943)*) also have made an extensive study of the factor. They first developed a chemical assay method for the factor which involves a two hour incubation of the fish brei or extract with thiamine in phosphate buffer at pH 7.0 and 37.5° C. After protein precipitation, aliquots of the filtrate are assayed for thiamine by a yeast fermentation method. Utilizing this method, the following information was obtained: (1) an active tissue powder can be prepared by drying the acetone-defatted tissue over phosphorus pentoxide, (2) the optimal activity of the factor occurs at pH 9.1 and 60°C., (3) first-order velocity constants are obtained for the rate of thiamine destruction by the factor, and (4) in the presence of excess thiamine, the velocity is proportional to the amount of the fish principle used.

It has been suggested in the past that the thiamine inactivation might be due to the formation of an inactive protein-thiamine complex. This suggestion has been offered largely out of analogy with the biotin-avidin complex. Sealock *et al.* attempted to hydrolyze the inactivated thiamine mixture by heat, acid, or proteolytic digestion and could find no evidence for this contention. All the available evidence indicates rather that the factor is a protein with enzymatic action. The products of the thiamine degradation, if such occurs, are unknown.

Sealock and co-workers found the antithiamine factor to be widely distributed in the various tissues, being highest in the spleen. Gastrointestinal and gill tissues were relatively high, while blood, kidney, heart, brain, testes, and ovaries were relatively low. None was detected in muscle or the swim bladder.

It has been thought that the toxic effects of raw fish could not be attributed to any particular species of fish since paralysis and death of foxes could be caused by feeding pike, carp, suckers, and many other types. Deutsch and Hasler (*Proc. Soc. Exp. Biol. Med.* **53,** *63 (1943)*) have found the factor present in 15 out of 31 species of fresh water fish examined, but in none of 9 species of salt water fish. Others however have reported it in Atlantic herring and whiting and in the Pacific mackerel. Where thiamine values of fish have been reported, as in many varieties of market fish, it is reasonable to believe that these do not contain the inactivating factor.

EATING UNDER WAR CONDITIONS

The pertinent problem of eating in wartime was the basis of a recent public lecture by Stare (*New Engl. J. Med.* **228,** *809 (1943)*). After an outline of general principles, Stare discusses the question, "How can an adequate protein supply be obtained with meat rationing?"

The weekly meat ration approximates 1.75 pounds or 840 g. Allowing about 20 per cent loss because of bone, 672 g. of meat remain. Since only about one fourth of this is actually protein, 168 g. of protein are available through the meat ration, or about 24 g. per day. This represents about one third of the protein intake recommended by the Food and Nutrition Board for an average adult.

The difference can be made up by using unrationed meats, fish, cheese, milk, vegetables, particularly beans. Peas, lentils, peanut butter, and the cooked cereals are also good sources of protein. It is stated that if unrationed meats were added to the ration list and even if the daily allowance were cut in half, one could still have a diet nutritionally adequate in protein provided there was a plentiful supply of the vegetable proteins and milk.

The public should be informed also about the factors which influence the nutritional quality of food. These include weather conditions and the type of soil on which the food is grown; the length of time from harvesting the food until it is processed; the length of time and the temperature of any blanching treatment or any process in which the food is in contact with water; contact with bright sunlight or unusual exposure to air, as would occur if the food were chopped or finely divided; the reaction of the food—that is, whether it is slightly acid or alkaline; and the degree and duration of heat used in processing the food.

It is necessary to educate the public to the fact that in home cooking excesses of heat and of water should be avoided, and that water added in cooking should be used again for soups or gravies. The alkalinity induced by using baking soda causes certain nutrients to be less stable. There should be a minimum amount of stirring; the pressure cooker represents an important step in the more efficient cooking of food. It is to be noted that the nutritive quality of food served in many restaurants is often inferior to that of home cooked food because it is frequently prepared several hours in advance and is kept warm, or is warmed over.

Research by the canning industry has been of great value in producing a canned food of nutritional quality which approaches that of the freshly harvested product. This is attained because the food is harvested primarily for canning, is brought directly to the factory, and is completely processed within three or four hours. Food obtained at markets for home canning may have been harvested several days earlier. Commercial canners, moreover, in most instances now appreciate the fact that the nutritional quality of food is better preserved if the food receives a short blanching with boiling water or steam, and is then cooked in a sealed container, which minimizes contact with the air. The cooking is usually at a high temperature and for a relatively short period.

Frozen foods likewise have a high nutritional quality for similar reasons. The public must be warned, however, that in the freezing process the cells are usually ruptured, and that when the food is warmed, there is a rapid loss of oxidizable and water soluble nutrients. Hence frozen foods should be cooked and consumed promptly after being thawed.

The dehydration of food is a process which is only developing. Much further study is necessary to establish the best technic for dehydration, keeping in mind not only loss of nutrients during storage, but also palatability of the end product.

STABILITY OF CAROTENE IN FORTIFIED FOODS

Recent interest in food fortification has led to attempts to fortify many foods, some of which have not as yet received the approval of federal officials or nutrition authorities. Before a fortification program can be approved in principle, it must be shown that the food under consideration is consumed in sufficient amounts by the general public so that its fortification or enrichment is likely to contribute to general improvement of public health. Furthermore, there is no advantage, so far as nutritional benefits are concerned, in fortifying foods with essential nutritive factors if these essential factors are unstable and are destroyed largely during storage or food processing.

With the last mentioned problem in mind, Morgal, Byers, and Miller of Michigan State College (*Ind. Eng. Chem.* **35,** *794 (1943)*) have studied the stability of β-carotene when added to certain oils, fats, and wheat and soybean flours. In order to obtain the maximum amount of information in the shortest possible time, it was necessary to devise an accelerated decomposition test for carotene. This was done by subjecting the foods fortified with carotene to temperatures of 45° and 60°C., respectively, in thermostatically controlled electric ovens for varying lengths of time. The carotene was said to contain 90 per cent of β-carotene and 10 per cent of α-carotene. Weighed amounts of carotene were dissolved in low boiling petroleum ether, known amounts of oil were added, and the petroleum ether was evaporated under reduced pressure at 50°C. in an atmosphere of nitrogen. Carotene analyses were made by the method of Petering, Wolman, and Hibbard (*Ind. Eng. Chem.* (*Anal. Ed.*) **12,** *148 (1940)*).

The first phase of the study dealt with the stability of crystalline carotene in oils at 45°C., with and without the presence of added antioxidants. Hydroquinone, phenyl-α-naphthylamine, phenyl-β-naphthylamine and α-tocopherol were added at levels of 0.01 per cent. Hydroquinone and α-tocopherol were tested for their antioxidant power at 0.10 per cent levels. Concentrates prepared from alfalfa were also tested as sources of carotene. Stability tests in this series were run for nearly five hundred hours.

Only hydroquinone was effective in retarding carotene destruction. The higher concentration (0.10 per cent) which was most effective permitted not more than 20 per cent loss of carotene in four hundred seventy-five hours. It was obvious that the natural antioxidants in the vegetable oils were themselves effective. In a "control" experiment, in which the carotene was dissolved in pure mineral oil, all the carotene had been destroyed in two hundred eighty hours at 45°C. This was in contrast to a 35 per cent destruction which occurred in corn oil under the same conditions.

One experiment is cited in which 89 per cent of the carotene in an alfalfa concentrate remained after eight hundred sixty-five hours, indicating that the natural antioxidants of alfalfa are even more efficient than hydroquinone at levels of 0.10 per cent. When the alfalfa concentrate is dissolved in corn oil, the protective effect is not additive, indicating that there is sufficient natural antioxidant in corn oil to stabilize the carotene. It is suggested that alfalfa concentrates should be more stable than crystalline carotene when added to foods relatively poor in natural antioxidants.

The second phase of the study had to do with the stability of carotene in edible fats. Corn oil concentrates containing crystalline carotene and alfalfa concentrate, respectively, were added to vitamin A-free samples of Crisco, lard, and oleomargarine in such concentration as to bring all fats to about 10,000 I.U. of vitamin A per pound. These were analyzed for carotene and then subjected to oxidation tests at 45°C. for from six hundred to one thousand hours. Some

stability tests were conducted for over six thousand hours at room temperature and at 8°C. in a refrigerator, using screw top sample bottles as containers.

It was concluded that Crisco and oleomargarine were superior to lard in their content of natural antioxidants. The addition of artificial antioxidants to these fats did not increase carotene stability. Carotene stability in lard was improved, however, when alfalfa concentrate was used instead of crystalline carotene. More than 90 per cent of added carotene remained when Crisco was heated at 45°C. for one thousand hours.

Similar studies were conducted with white bleached flour and soybean flours in which alfalfa concentrate and crystalline carotene were added to the amount of 60,000 I.U. of vitamin A per pound of flour. It was found that carotene is relatively stable in solvent extracted soybean flour, from 81 to 96 per cent of the carotene remaining after one hundred sixty-nine days at 45°C. Lecithin improved the stability of carotene in soybean flours. Carotene stability in bleached wheat flour was relatively poor, even when the carotene was added in the form of alfalfa concentrate.

Considerably more information is needed before it will be possible to recommend indiscriminate fortification of foods with provitamin A and vitamin A. It is interesting to note, however, that oleomargarine tends to stabilize carotene. Fortification of this fat with vitamin A has already received the approval of the Food and Nutrition Board of the National Research Council and federal food authorities.

RELATIONSHIP BETWEEN VITAMIN B-COMPLEX INTAKE AND FATIGUE

Several studies on the relation of vitamin intake to physical fatigue due to hard muscular work were reviewed recently (*Nutrition Reviews* **1**, *227* (*1943*)). An additional report by Barborka, Foltz, and Ivy has just appeared (*J. Am. Med. Assn.* **122**, *717* (*1943*)).

In this study 4 medical students fed a normal adequate diet were trained for nine to twelve months to work an electrodynamic brake bicycle ergometer. This was done three times a week at a rate of 1235 kilogram-meters a minute with a pedaling rate of 54 revolutions a minute. In order to reduce the error in this method of measurement, the subjects worked to complete fatigue, rested ten minutes, and then worked again to complete fatigue.

After this prolonged training period, the students were told that they were being put on a high carbohydrate diet, but were unaware that they were to receive a diet deficient in vitamins of the B-complex. The thiamine content of the modified diet was reduced from 1.80 to 0.65 mg. daily, and the riboflavin from 2.70 to 0.94 mg. There was also a reduction in the vitamin A, ascorbic acid, calcium, phosphorus, and iron content of the new diet, amounting to almost 50 per cent.

After two months on this regimen, half of the fat calories were replaced by isocaloric amounts of carbohydrate. After three weeks of this modification, the students were returned to the previous deficient diet, but this was supplemented with a yeast concentrate given in lemon or tomato juice. In this way the thiamine content of the diet was increased 15 mg., the riboflavin 5.25 mg., and other B-vitamins were added. Furthermore, the vitamin A and ascorbic acid contents were increased substantially.

During the period on the deficient diet, the subjects developed irritability, easy fatigability, lack of energy, anorexia, and increased leg pain during the work periods. At no time did they develop any objective physical signs of vitamin B-complex defi-

ciency, although the twenty-four hour urinary excretion of thiamine and riboflavin dropped to low levels. The blood pyruvic acid, as well as the blood thiamine, remained in the normal ranges during the course of the deficiency periods.

The graph representing work done reveals a rapid increase in amount of work during the early part of the training period, and a small fluctuation thereafter. During the period of the deficient diet there was a gradual decrease in amount of work done. When the fat content of the diet was reduced, there was no change. When yeast concentrate in lemon and tomato juice was added, there was a sharp increase in work done, which tended to fall off after a short period of time. The authors conclude that vitamin B-complex added to a vitamin B-complex inadequate diet restores work output to efficient levels.

The results would have been more convincing if the period of observation after an adequate diet was restored had been longer than four weeks, thus making better comparison possible with the preliminary training period. However it should be stated that these experiments, in contrast with previous work on the relation of nutrition to fatigue, were well controlled with respect to the length of the training period and the fact that the subjects were unaware of their actual nutritional status. It is extremely difficult to rule out the psychologic factors in such a test, when the subjects know that their diets have been changed—especially when it has been made more attractive. One might question whether the decreased work output was due solely to changes in the amount of vitamins of the B-complex, since the amount of other nutrients also changed. Vitamin A, ascorbic acid, calcium, and phosphorus decreased some 50 per cent on the deficient diets. It must be pointed out, however, that the levels of vitamin A and ascorbic acid on the deficient diets were above those generally recommended. Previous research has shown that a superabundance of vitamins does not lead to greater work output.

This work gives good support for the view that an inadequate diet reduces the individual's efficiency without causing effects which would be readily recognized by the usual methods of observation. This, moreover, is more likely to occur in persons who do strenuous physical work. How important all of this is from the standpoint of national economy depends largely on the extent of nutritional deficiency which exists throughout the country. Some competent observers claim that at least one third of the American population is eating a diet which is not adequate in all respects. It must be emphasized, however, that it is only in those who are taking inadequate diets that improved efficiency of work and a sense of well-being can be expected through improvement of the diet.

INCREASED ERYTHROCYTE DESTRUCTION ON A HIGH FAT DIET

The hemolytic properties of soaps *in vitro* have long been known, but only recently has good evidence been provided that a similar action may occur in the blood stream. Johnson and Freeman (*Am. J. Physiol.* **124,** *466 (1938)*) demonstrated that lymph samples collected from dogs following fat ingestion are markedly hemolytic; over half of the erythrocytes in samples of heparinized blood may be destroyed by mixing the blood with fatty chyle. These observations raise the question of whether hemolysis might occur in the blood stream following fat ingestion. Investigations in two different laboratories indicate a positive answer to this question.

Loewy, Freeman, Marchello, and Johnson (*Am. J. Physiol.* **138,** *230 (1943)*) prepared

both internal bile-fistula dogs (so that the total bile output was diverted to the urinary bladder and excreted in the urine) and external bile-fistula dogs. When the animals were in good clinical condition and gaining weight, daily collections of twenty-four hour urine-bile and bile samples were begun. Bilirubin determinations were made daily by a modification of the procedure of Malloy and Evelyn. All experimental animals were maintained on a basal diet of supplemented canned commercial dog food plus a daily dose of 0.5 g. of pure sodium taurocholate. The "high fat" diet consisted of this basal diet plus 5.0 to 10.0 ml. per kilogram of body weight of olive or corn oil. Cane sugar was substituted for the oil in calorically equivalent amounts to make the high carbohydrate diet. Most of the dogs were continued upon the experiment for several months with successive changes from one diet to another after periods of from eight days to three weeks.

With each change from basal diet to high fat diet the bile pigment output increased and in turn decreased when the animals were returned to the basal diet. Bile pigment excretion was greater on the high fat diet than on the calorically equivalent high carbohydrate diet. Thus, the excretion of extra bile pigments appeared to result from an increased red cell destruction following fat ingestion and not from an increased caloric intake. Bile pigment excretion, in terms of the average output on the basal diet, averaged 137 per cent on the high fat diet, a statistically significant difference. Similar results were obtained with both the internal and external bile-fistula animals.

Since no significant anemia developed despite the increased red blood cell destruction, apparently normally functioning hematopoietic tissue can compensate for the extra losses induced by fat feeding. The authors suggest that red cell destruction by chyle may be one mechanism for normal red blood cell destruction in the dog.

Josephs, Holt, Tidwell, and Kajdi (*Bull. Johns Hopkins Hosp.* **71,** *84 (1942)*) studied the urobilin excretion of 6 normal infants between 5 and 8 months of age. Various fats (butterfat, goat's milk fat, human body fat, corn oil, olive oil, and soybean oil) were incorporated in a skim milk diet by homogenization. Diets to which free fatty acids and soaps had been added were also studied. The quantity of fat ingested was varied, between 35 and 85 per cent of the calories being supplied in this form.

The infants were kept upon metabolism frames to ensure complete collection of the excreta. Urobilin determinations were made by the method of Elman and McMaster. It was found that significant variations in the urinary urobilin did not occur and these determinations were discontinued. The stools were collected and analyzed daily but because of diurnal variations the results were reported as averages for two or three days at a time.

An increase in the fat of the diet produced a constant and significant rise in urobilin excretion. All the animal and vegetable fats studied seemed to produce about an equal increase in urobilin output. When the fat was increased from 35 to 85 per cent of the caloric intake, the urobilin excretion rose from 15 or 20 mg. a day to 30 or 40 mg. A relatively small addition of either free fatty acid or soap to the diet (10 per cent of the caloric intake) produced a rise in fecal urobilin output comparable to that brought about by a large increase in the dietary fat.

The presence of extra fat in the intestines of infants on a high fat diet might interfere with the reabsorption of bile pigment from the intestine and result in an increased fecal urobilin output, but this explanation does not seem to fit the facts. If solubility of urobilin in the intestinal fat were responsible for diverting urobilin to the stools, then the feces should show proportional increases in fat and urobilin. However, the fat content of the feces from the high fat diets was within the normal limits for infants on the usual levels of fat intake. In one experiment 5 g.

of mineral oil were added to a normal diet in order to test the possible effect of the solubility of urobilin in fecal fat. The feces from this diet contained roughly twice as much oily material as from any of the other diets studied. The addition of mineral oil produced a definite temporary increase in urobilin output, but the excretion returned promptly to its original level. If the increased excretion had been the result of diversion of dissolved urobilin, it should have continued as long as the mineral oil supplement was continued.

It is well known that fats cause an evacuation of the gallbladder but the increased urobilin output can hardly be explained upon the basis of "washing out" the stores of bile pigment. No doubt the initial high rise in urobilin output is partially, at least, the result of an increased bile flow, but the sustained output (in 2 subjects for more than two weeks) above the normal level requires some other explanation. The authors conclude that the increased urobilin output from a high fat diet must be attributed largely to increased blood destruction.

In view of the known hemolytic action of soaps and the fact that small amounts of either free fatty acids or soaps are as effective as large amounts of fat in increasing the urobilin output, the authors suggest that the hemolytic effects of high fat diets are the result of increased absorption of soaps as such.

These two papers clearly indicate that, in the dog and human infant at least, high fat diets lead to an increased rate of red blood cell destruction. However, since this process does not produce an anemia, it is difficult to evaluate the importance of these findings. The results suggest that individuals with bone marrow involvement should avoid high fat diets because of the extra burden placed upon the hematopoietic tissues. Additional observations under various clinical conditions will be necessary before these findings have practical application.

EFFECT OF CARROTS ON THE RESISTANCE OF RATS TO ACUTE ANOXIA

The problem of increasing the resistance of man to high altitudes and low oxygen pressures is obviously of paramount importance to aviation. The announcement several years ago, therefore, by Campbell (*Quart. J. Exp. Physiol.* **28,** *231 (1938)*) that a diet of carrots would protect rats from the effects of acute anoxia was of considerable interest. Confirmation of the observation by Nelson, Goetzl, Robins, and Ivy (*Proc. Soc. Exp. Biol. Med.* **52,** *1 (1943)*) has served to reemphasize its possible importance.

Campbell noted that exposure of rats to a barometric pressure of 240 to 250 mm. Hg. in a small decompression chamber at a temperature of 33°C. led to death of the animals within ten minutes. Diets of horse meat, milk, salmon, and fresh liver offered no protection against these low pressures, but a pure carrot diet afforded definite relief. After one day of carrot feeding, 12.5 per cent of the rats withstood the exposure for thirty-five minutes while after six days all animals survived the test. Parsnips and beetroot also gave some protection but not so much as that given by carrots. The carrot effect was reduced when half the carrot was replaced by other food substances. Boiling the carrots for fifteen minutes did not lower their protective value and it was shown that the important factor was present in the fiber and more solid portion rather than in the juice. Since rats on the carrot diet lost 5 to 10 per cent of their body weight in six days of carrot feeding, it was thought that weight loss alone might be responsible for some of the beneficial effect. However, animals subjected to starvation so that they lost a comparable amount of weight were not more resistant to low barometric pressures. A synthetic nonprotein diet with almost no fat and containing rice-starch 70.5 per cent,

yeast 3 per cent, salts 1.5 per cent, and cellulose 25 per cent gave some protection. The cellulose in the diet seemed important since the effect was lost when cellulose was replaced by kaolin or agar. Cellulose was not the only factor involved, however, because the cellulose content of the parsnips consumed was equal to that in the carrots, yet parsnips were not as effective. Yeast did not influence the results. There is, therefore, some substance in carrots which opposes oxygen want. How this substance exerts its effect (or its nature) was not discovered.

In a continuation of his studies, Campbell (*Quart. J. Exp. Physiol.* **29,** *259 (1939)*) demonstrated that bananas and apples also had a carrot-like effect. Zein was the only protein studied which may constitute 50 per cent of the diet and give protection against oxygen want. Gelatin was partially effective but casein and meat increased susceptibility to oxygen deficiency. An analysis of individual amino acids showed that tryptophane, tyrosine, lysine, and glutamic acid had beneficial effects while histidine and arginine were unfavorable to oxygen lack. Campbell concluded that at least four factors are responsible for the beneficial action of carrots: (1) low protein content (1 per cent), (2) presence of fiber, (3) presence of glutamine, and (4) an unknown factor. He suggested that fiber might act in the intestinal tract as an adsorbent since the inclusion of charcoal and paper pulp in the synthetic diet also gave some partial protection.

Confirmation of these results by Nelson and associates was limited to the effect of a carrot diet. Two hundred and fourteen male rats were divided into two equal groups; one group was fed nothing but fresh carrots while the other was given a standard kennel ration plus ground corn, whole milk, bread, and lettuce. On the eleventh day the animals were subjected to a pressure of 225.6 mm. Hg. (simulated altitude of 30,000 feet) for two hours at a temperature which ranged from 78°F. to 86°F. Of the carrot fed rats, 79 per cent survived the exposure while only 21 per cent of the control animals lived. No attempt to define the mechanism of the protective action of carrots was made.

Nelson cautions that it may not be valid to draw conclusions from data obtained under one condition of anoxia as to what would obtain in more or less severe degrees of oxygen want or exposure to other altitudes. Certainly it is not clear that the results of these experiments will have value in practical problems of high altitude flying. Rats are known to be markedly different from man in their response to altitude exposure. Nevertheless, the mechanism of the carrot effect promises to be highly interesting when it finally is elucidated.

METABOLISM OF ZINC

The element zinc is essential for the growth of rats and must also be required by man since it is known to be a constituent of the enzyme, carbonic anhydrase. The requirements are undoubtedly small and the element is widely distributed in foods. Egg yolk, meat, whole wheat, and particularly yeast and oysters are rich sources of zinc. Attention has been directed to the metabolism of zinc in the past more from the standpoint of toxicologic effects because of its use in industry than from a nutritional standpoint. More recently the daily use of zinc protamine insulin by diabetics has undoubtedly aroused additional curiosity in the metabolism of zinc. Reports indicated that the usual human intake is approximately 15 mg. per day and that this would actually be increased by only 0.1 to 0.2 mg. by the use of zinc insulin preparations. The earlier work of Drinker, Fehnel, and Marsh (*J. Biol. Chem.* **72,** *375 (1927)*) has already

shown that after a meal rich in zinc, the urinary excretion of zinc increases very little, although much additional zinc is excreted in the feces.

McCance and Widdowson (*Biochem. J.* **36,** *692 (1942)*) have experimented further on the metabolism of zinc by conducting balance studies on 12 individuals. The period of study on each level of zinc intake lasted for two weeks. Low zinc intakes (5 mg. per day) required special planning of the diet, moderate intakes (10 to 14 mg. per day) were supplied when white flour constituted nearly half of the total calories, and high intakes (20 to 22 mg. per day) were provided by including 92 per cent extraction wheat meal in the diet. The subjects were found to be in zinc balance at all of these levels. There was no increased urinary output of zinc due to a higher intake; the higher excretion occurred in the feces. Zinc therefore resembles iron in that the kidneys seem to exert little or no regulatory control on the amount of the metal in the body. The absolute amount of zinc excreted in the urine per day was approximately 0.3 mg.

When additional zinc salts were administered intravenously in amounts ranging from 58 to 86 mg. of zinc in divided doses and over the course of two weeks, the subjects again established balance promptly and without change in the amount excreted in the urine. The authors suggest that the constant trace of zinc found in the urine may represent only a metabolic function of the kidney itself. The zinc may well be closely associated with plasma protein, a reasonable explanation for failure of extra zinc excretion in the urine when the plasma level is increased.

The latter thought led the investigators to examine zinc excretion in patients with moderate or severe albuminuria. They noted that such patients excreted much larger quantities of zinc in the urine, ranging from 1.0 to 3.8 mg. per day, although the amount of zinc was not well correlated with the actual degree of albuminuria. Balance studies on two patients with heavy albuminuria revealed that these subjects were in a negative zinc balance. Instead of a lessened excretion of zinc in the feces to compensate for the greater urinary output, the fecal excretion was essentially equal to the intake. The authors concluded that such patients do not absorb sufficient zinc from ordinary diets to compensate for the urinary losses. Such individuals, according to these data, should develop a zinc deficiency, but symptoms of such a deficiency are unknown.

More recently, Sheline, Chaikoff, Jones, and Montgomery (*J. Biol. Chem.* **147,** *409 (1943)*) have attacked the problems of zinc metabolism with the aid of its radioactive isotope, Zn^{65}. The use of this material and the sensitive method of analysis provided by the Geiger counter made possible the use of minute amounts of the element. Approximately 0.4 microgram of zinc was injected into mice and approximately 6 micrograms into dogs. The total zinc content of the body is not increased appreciably by this amount of labeled zinc, hence the authors believe that the excretion of Zn^{65} forms a true picture of normal endogenous zinc excretion. They found that a large portion of the Zn^{65} appeared in the feces. This amounted to 50 per cent of the injected quantity in seven to eight days in the mice, and approximately 25 per cent in twelve to fourteen days in the dog. Although the radioactive zinc appeared early in the urine, less than 5 per cent of the total was excreted by this route in the above periods of observation.

The distribution of radioactive zinc in the tissues after intravenous injection was also studied by Sheline, Chaikoff, Jones, and Montgomery (*J. Biol. Chem.* **149,** *139 (1943)*). They noted a rapid disappearance of Zn^{65} from the plasma of the dog and early deposition in the liver. Three hours after injection, the dog's liver contained 38 per cent of the total Zn^{65}. The "turnover," that is, the rapidity of uptake and loss, was greatest in the liver, pancreas, kidney, and pituitary gland. The slowest turnover occurred in the red blood cells, brain, skeletal muscle, and skin. Spleen, gastrointestinal

tract, adrenals, lungs, bone, heart, and thymus were intermediate in this respect.

These studies show a wide distribution of zinc in the animal body. In addition to the presence of zinc in carbonic anhydrase, the metal has been found in purified uricase preparations and appears to be necessary for the storage of insulin in the pancreas. The evidence indicates that the zinc of the body is widely distributed and is probably associated with proteins, at least some of which are enzyme molecules.

PANTOTHENIC ACID AND REPRODUCTION

Students of practical therapeutics have always had to struggle against the natural tendency to believe that if a given amount of any substance has therapeutic value, greater quantities of the same material will accomplish even more. This feeling has applied particularly to the vitamins. It has received encouragement from isolated clinical impressions like those which claim that large doses of vitamin D or of the B-complex may favorably influence the course of patients with some forms of arthritis. However, carefully controlled experiments with animals have, in general, indicated that the health and nutritional status of animals is not improved when they are given more than the established optimal amounts of individual vitamins. For this reason it is important to take cognizance of the recent demonstrations that pantothenic acid in excess of the daily requirement appears to influence the rate of reproduction at least in fowl, rats, and mice.

Taylor and associates (*Science* **94,** *542 (1941)*; *Univ. Texas Publ. no.* **4137,** *67 (1941)*) found that increasing the pantothenic acid available to developing chick embryos led to a greater hatchability of eggs. However, the relative sizes of the brain and heart decreased in those embryos which developed from the pantothenic acid enriched eggs. The pantothenic acid content of the eggs was increased either by direct injection into them of 100 to 150 micrograms of pantothenic acid or by supplementing the diet of a flock of hens with three times the daily requirement of this vitamin.

Because of the special conditions of food supply available to oviparous embryos, Taylor, Pennington, and Thacker have extended their observations to include the effect of calcium pantothenate supplements on the reproduction of viviparous animals (*J. Nutrition* **25,** *389 (1943)*). Albino rats of the Wistar strain and mice of the dba strain were used in these studies. In the first experiment, 60 breeding female rats about 5 months old were divided into two groups. The controls were fed Purina dog chow which supplies 14 micrograms of pantothenic acid per gram. The test animals were maintained on the dog chow supplemented with 100 micrograms of calcium pantothenate per gram of ration. After two weeks, males were introduced to both groups. Observations were continued for a period of fifty days. The second experiment was comparable in all respects to the first except that mice, about 3 weeks old, were used. The third experiment, continued for thirty-three days, was also done on mice but the test animals were given their supplements of pantothenic acid by subdermal injection of 400 micrograms daily. Control mice were injected with saline alone. The animals which received the added pantothenic acid showed an increase of 21 to 27 per cent in the size of the litters in all three experiments. The weights of the brain and heart, determined only on the 1 day old rats, were approximately 14 per cent less in the young born of mothers fed the calcium pantothenate supplement. These results, therefore, confirm those which had been obtained earlier on chicks.

Taylor and associates believe that pantothenic acid produces its effect on litter size by increasing the survival of fertilized ova rather than by changing the number of eggs

produced by the ovaries. The results appear to be statistically significant. They demonstrate that at least this one vitamin has a physiologic effect in large doses which is not shown by the amount necessary to maintain health. Taylor does not evaluate the effect as a desirable one, but there is danger that it might automatically be so considered. However, since individual organs decrease in weight, one wonders whether the subsequent performance and life expectancy of these animals will be as good as that for young born of mothers not given the added amounts of pantothenic acid. More information must be acquired before it can be decided whether the effect produced in these experiments should be avoided or cultivated.

VITAMIN E AND NEUROMUSCULAR REGENERATION

Numerous investigators have observed that diets deficient in vitamin E lead to degeneration, paralysis, and weakness of the skeletal muscles in various species of laboratory animals. Tocopherol is effective in preventing this degenerative process or in curing it if the degeneration has not proceeded too far. This curative effect suggests the possibility of tocopherol assisting in muscle regeneration following other types of destructive lesions.

Hines, Lazere, Thomson, and Cretzmeyer (*Am. J. Physiol.* **139,** *183* (*1943*)) have reported a study of the extent and velocity of denervation atrophy of muscle and subsequent neuromuscular regeneration using rats and guinea pigs as experimental subjects. Comparisons were made of the changes occurring in animals maintained on three different levels of vitamin E intake (E deficient, adequate, and adequate plus excess vitamin E). Complete denervation of the gastrocnemius muscle was accomplished by crushing the tibial nerve at the point of its junction with the peroneal. Electrical stimulation of the nerve above the lesion immediately after the crushing did not produce muscular contraction, thus demonstrating complete denervation. Electrical stimulation of the nerve below the lesion three days after the operation also failed to produce muscular contraction, indicating that degenerative changes had occurred. Muscle strength, weight, creatine concentration, and the capacity of the nerve to activate its muscles were determined at various intervals following the operation and compared with similar findings from the contralateral limb which was not operated upon.

In previous experiments (*Am. J. Physiol.* **137,** *527* (*1942*)) it was reported that the degenerative changes resulting from this operation (in the rat) occur most rapidly during the first two weeks followed by progressive recovery beginning after about three weeks. It is now reported that the loss in muscle strength and weight during the two weeks' period was somewhat greater in the vitamin E deficient animals than in their adequate diet controls. However, muscle regeneration as judged by muscle strength and mass occurred at a comparable rate in both E deficient and adequate diet animals. Furthermore, the rate of nerve regeneration, as judged by its capacity to activate muscle, was not improved by an excess of vitamin E. Similar findings were reported from the guinea pig studies.

Under the conditions of these experiments, the quantity of vitamin E in the diet, therefore had little effect upon the extent or velocity of neuromuscular regeneration. Furthermore, if vitamin E is at all necessary for neuromuscular regeneration, the requirements are so small as to be supplied by an "E-deficient diet." These findings suggest the futility of supplying large amounts of vitamin E to aid in the recovery of muscle and nerve from lesions other than those actually arising from vitamin E deficiency.

NOTES

The Spread of the Potato—Part II

About ten years before Count Rumford's successful battle for the spread of the potato in Bavaria a less prominent person fought for the new food in France. Until 1790 the potato was a rare and an exclusively royal delicacy in France. But in spite of such prestige it failed to become popular. A few years before the French revolution an army apothecary named Parmentier attached to the French military hospitals stationed in invaded regions of Germany observed the use of the potato in some localities and wished to see it gain favor in his homeland. The potato was not very popular in Germany either at the time, but it had gained a foothold here and there. In the year 1749 an observer wrote that the potato was "considered (in Germany) the worst vegetable though the very poorest are obliged to eat it." In 1761 Turgot, the French political leader, sought to popularize it among his country's impoverished and starved peasantry. That there was need for a new food is evidenced by the fact that the French Academy offered a prize for any new item that could relieve starvation.

It was then that Parmentier began his campaign in earnest. Marie Antoinette and Louis XVI gave him land in which he planted potatoes. The king wore potato blossoms in his lapel and Marie Antoinette in her hair. The courtiers ate and praised the potato at court. Yet the peasantry stood aloof.

Parmentier sought to sell potatoes cheap and found no buyers. The following year he gathered in a big crop and offered it free of charge to all farmers, but still they would not buy. In view of the fact that a few landowners had planted it on their estates, he hit on a simple ruse. He ordered town criers in all villages to announce with special pomp and circumstance that the theft of potatoes in the field by those who were curious to try out this wonderful fruit would be severely punished. This resulted in much pillage and many peasants began raising potatoes.

It is of interest that practically every German duchy or kingdom had its potato apostle who proselyted and agitated for its spread. Country preachers who were more farsighted than the farmers and advocated its acceptance were popularly known as "potato preachers." But once the hurdle of initial resistance was crossed, the crop was recognized everywhere as a blessing. It may now well be regarded as the savior of the poor in Europe since it yields more food per acre than any other starch crop.

Soybean Symposium

A symposium on soybeans appeared recently (*J. Home Econ.* **35,** *201 (1943)*). Dr. Salmon of the Alabama Experiment Station discussed "Soybeans for Human Food." A serving of 100 g. of mature soybeans will supply 40 per cent of the protein, 25 per cent of the calcium, and over 60 per cent of the iron required by the adult for one day. Like most vegetables, soybeans are low in riboflavin and nicotinic acid. Mrs. Jane Hale of the University of Tennessee continued with "Soybeans in the Diet" and offered recipes. Dr. Simpson, University of Illinois, reported "Soybean Studies in Illinois" —varieties, types, and uses. Finally, the "Effect of Different Cooking Methods on Soybean Proteins" was presented by Dr. Helen Parsons of the University of Wisconsin.

An interesting booklet on soybean food preparation has been prepared for distribution by the School of Nutrition, Cornell University.

Carotenemia in a Nursing Infant

Another instance of carotenemia in a nursing infant of a mother who consumed regularly large amounts of carrots has been

reported by Thompson (*Arch. Dis. Childhood* **18,** *112* (*1943*)). The infant was brought by its mother to a physician at the age of 2 months because of the development of a jaundice-like condition in both mother and child. Breast feeding was stopped and the condition of the baby cleared, but that of the mother did not. Three months later on again consulting a physician it developed that the mother had for nearly a year been in the habit of eating from two to three pounds of carrots each week. At this time the child was plump and well nourished and carotene could no longer be detected in large amounts in the blood, thus emphasizing again (*Nutrition Reviews* **1,** *105* (*1943*)) that carotenemia of itself is not harmful even though the discoloration of the skin may be alarming and may at times be confusing to the diagnostician.

Mineral and Vitamin Values of Foods

A second compilation of recent data on mineral and vitamin values of foods has been prepared by a subcommittee of the Diet Therapy Section of the American Dietetic Association under the chairmanship of Dr. Ruth Cowan Clouse (*J. Am. Dietet. Assn.* **19,** *496* (*1943*)).

Forty-nine articles have been reviewed covering the period from May 1942 to December 1942. An examination of these data shows that in addition to continued study of the vitamin B-complex, much research has been directed toward analyses of fruits and vegetables for the vitamin C content with particular reference to the effect of cooking and other methods of processing on this vitamin.

A New Food Yeast

Under the auspices of the Colonial Development Board of Great Britain a sum of approximately $125,000 has been granted for the erection in Jamaica of a plant capable of producing annually 2000 tons of a new dehydrated yeast. The yeast is known as *Torula utilis* and it is closely related to the ordinary brewers' yeast and has similar nutritive values. There has been developed a diploid strain by growing the normal organism in media containing camphor. The strain is nonspore forming and has been called *T. utilis grande* because the size is twice that of the normal haploid strain. This yeast is grown on black strap molasses with added quantities of ammonium sulfate and ammonium phosphate under aerobic conditions with careful control of temperature and pH values. A continuous process has been developed, largely by A. C. Thaysen and collaborators, in which fresh wort is run in at the top of the vat and fermented liquor containing the yeast is removed from the bottom. The yeast is centrifuged, washed, and then dried by the roller process to yield a pale yellow flake of inactivated yeast. In an article describing the process (*Food Manufacture* **18,** *117* (*1943*)), it is stated that "there should be no reason why further plants should not be put up in other West Indian islands" in order to utilize waste molasses, sugar, and bananas.

Modified Standards for Flour Enrichment

The Federal Register dated July 3, 1943, sets forth modified requirements for flour enrichment. This document can be obtained at a cost of ten cents by writing to the Superintendent of Documents, Government Printing Office, Washington, D. C. These requirements become effective October 1, 1943.

Riboflavin limits are now set at not less than 1.2 or more than 1.5 mg. per pound of flour, while the thiamine range is from 2.0 to 2.5 mg. per pound. The lower and higher limits for niacin (or its amide) are 16.0 and 20 mg. per pound of flour while iron (Fe) cannot be less than 13.0 mg. or more than 16.5 mg. For self-rising flours calcium supplement limitations are set at from 500 to 1500 mg. per pound.